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STUDY OF THE INFECTIVITY OF NUCLEIC ACIDS IN INSECT TISSUE CULTURE

(Experiments with the DNA-containing virus of nuclear polyhedrosis of silkworms)

[Following is the translation of an article by V. D. Miloserdova, Institute of Microbiology and Virology, AN UkSSR, Kiev, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology) No 4, 1965, pages 417-420. It was submitted on 15 Apr 1964.]

In the last 5 years many reports have appeared in the literature concerning the investigations of the infectivity of preparations of viral nucleic acids in tissue cultures of vertebrates. At first many authors could not achieve success in the contamination of tissue cultures by means of direct introduction of nucleic acids which were isolated from viral material. Treatment of the tissue cultures with hypertonic saline solutions prior to the administration of preparations of infectious nucleic acids promoted contamination and increased the titers of infectivity in a dependence on an increase in the concentration of the solutions. Thus O. G. Andzhaparidze and associates detected a positive influence of a 1 M solution of NaCl on the manifestation of a cytopathic effect of RNA from the virus of tick-borne encephalitis in tissue cultures of vertebrates.

Similar investigations were also conducted on tissue cultures of plants. Hirth and Lebourier were able to cause the infection of plant cells in a culture of infectious RNA from the tobacco mosaic virus. The authors established that the infectivity of RNA was 100-200 times lower than the infectivity of the tobacco mosaic virus itself [5,6].

The possibility of the production of the virus in insects with the help of RNA preparations from a host which was infected with the DNA-type virus was first demonstrated by S. M. Gershenzon and associates in 1960 in experiments with the chrysalis and larvae of bombyx [1]. The authors isolated RNA from bombyx caterpillars which were infected with nuclear polyhedrosis and administered it to healthy caterpillars, as a result of which the latter became ill with jaundice.

The aim of our investigations was a study of the infectivity of nucleic acids (DNA and RNA), isolated from jaundice infected larvae and chrysalises of bombyx, and also the infectivity of pure virus of nuclear polyhedrosis in insect tissue cultures.

In the tests we used tissue cultures of silkworm ovaries, taken from the stage of early chrysalis, seminal cysts from larvae and

chrysallises, and also cultures of blood cells from silkworm larvae.

As the cultural medium we used the synthetic amino acid medium of Grace * with the addition of a protein blood substitute instead of hemolymph [3].

* The amino acid medium prepared by us differed from Grace's medium by the fact that instead of levorotatory we used a double amount of racemic amino acids.

Materials and Methods

The silkworm chrysallises were initially sterilized in 60° alcohol for 10 minutes and then in 96° alcohol and dried with sterile filter paper. With sterile scissors the integument was cut from the dorsal surface and the ovaries were extracted. Initially they were placed in Puck solution [7], then rinsed three times in this solution and then in nutrient medium.

After washing, the tubules were extracted from the ovaries with dissecting needles and treated with trypsin.

The trypsin was prepared on Puck solution in a concentration of 1.5%, sterilized by means of filtration through a Seitz filter, and preserved in a frozen state. Prior to use the trypsin was diluted 10 times with this same solution and tissues of ovary tubules, cut into pieces 2-3 mm in size, were placed in it. They were maintained in the solution of trypsin for 30 minutes, all the time being mixed by hand or on a magnetic mixer. Then they were centrifuged for 2-3 minutes at 1500 rpm; the cellular precipitate was rinsed with cultural medium and the cultural suspension was prepared. A drop of cell suspension was placed on a cover glass and cultivated in hanging drop over the hole in the slide. The cover glass was attached to the slide with a mixture of paraffin and wax.

The suspension of cells of seminal cysts was prepared without treatment with trypsin, since during cutting of the testes the cysts themselves ran out and thorough mixing, with the help of a pipette, of fragments of testes with nutrient medium was fully sufficient for obtaining a cell suspension. The latter was also cultivated by the hanging drop method.

For the preparation of cultures of hemocytes blood was collected from surface sterilized caterpillars into sterile test tubes and centrifuged for no less than 5 minutes at 2000 rpm.

The fluid was decanted and the precipitate of cells was rinsed several times with cultural medium, on which the suspension of hemocytes was prepared.

As a rule the cultures for experiments on studying the infectivity of preparations of nucleic acids were prepared the day before the experiment was set up.

Preparations of nucleic acids (DNA and RNA) were isolated by the biochemistry group in our laboratory under the guidance of I. P. Kok. ** These acids were isolated by the phenol method from silkworm larvae and chrysalises, which were infected with the nuclear polyhedrosis virus, on the 4th-5th day after infection. The concentration of RNA in the preparations comprised basically 0.3%, and the concentration of DNA was various - from 0.05 up to 0.1%.

** We take this opportunity to thank I. P. Kok, I. N. Skuratovskaya, T. I. Bilaya, I. P. Stasovskaya, and G. N. Dobrovolskaya for kindly giving us the preparations of infectious nucleic acids.

Isolation of pure virus from inclusion bodies of the nuclear polyhedrosis virus was performed by the method of Bergold [4].

Prior to administration of the preparations of DNA and RNA the cultures were washed in some cases with Ringer solution, in others with a hypertonic solution of NaCl, and sometimes with nutrient medium or a suspension of bentonite. Such treatment was performed for the purpose of removing desoxyribonuclease and ribonuclease which had accumulated as a result of the vital activity of cultural cells. After treatment with one of the above-mentioned solutions the cultures were rinsed with nutrient medium for removing traces of solution.

Immediately after isolation a drop of the preparation of nucleic acid was introduced into the culture and the same amount of fresh cultural medium was added for the nourishment of the cells.

In all the experiments we used 6-7 cultures each, and each time an experiment was set up 1-2 cultures were infected with infectious hemolymph, obtained from infected silkworm larvae, as a control . We also left 2 control cultures in which Ringer solution was introduced.

In each of the series of experiments for studying the infectivity of purified nuclear polyhedrosis virus we used 8 cultures: in 2 cultures we introduced a suspension of virus particles in distilled water; for the infection of 3 cultures this suspension was diluted in a ratio of 1:1 with inactivated hemolymph, obtained from healthy silkworm chrysalises; 3 cultures were infected with pure virus, diluted with this same hemolymph in a ratio of 1:3.

At the same time we set up 4 control cultures, in 2 of which we introduced Ringer solution, and in 2 - hemolymph from an infected insect. Experimental and control cultures were incubated at 28-29° and the medium in them was changed regularly one time a week.

Results and Discussion

10 series of tests were set up for studying the infectivity of DNA and 10 series for RNA, and also 2 series of tests on the infection of insect tissue cultures with purified nuclear polyhedrosis virus.

The test cultures were followed for 2 months by means of microscopic observation for the appearance of polyhedra. Not in one of the 20 series of tests were we able to detect the development of nuclear polyhedrosis in cultures following the administration of preparations of nucleic acids or following their infection with purified virus particles.

In the control cultures, in which the hemolymph of an infected insect was introduced, in the majority of cases polyhedra appeared.

The negative results obtained by us in the experiments on the infection of insect tissue cultures with purified virus confirm the earlier experiments of the Canadian authors Vaughn and Faulkner [8]. Subsequently these investigators were able to cause the infection of cells and tissue cultures with pure virus particles only following the addition of polyhedrosis protein to the latter. In the opinion of the authors, polyhedrosis protein is the stimulating agent which promotes the penetration of virus particles into the cell. Up until the present time the infectivity of viral nucleic acids in insect tissue cultures had not been studied. The failure of both viral nucleic acids and purified virus to infect insect tissue cultures confirms the opinion of Faulkner and Vaughn that the penetration of pure virus particles, and also, apparently, large molecules of nucleic acids, into cells under cultural conditions requires yet some other factor which carries out the specific function. This probably explains the infectivity of hemolymph from an infected insect. It contains both virus particles and polyhedrosis protein, which in this case apparently plays an important role.

The negative results of infection of insect tissue cultures with RNA from a polyhedrosis infected host are possibly connected with the fact that during the isolation of RNA there is the possibility of its partial destruction, as a result of which it loses its ability to penetrate into a cell under cultural conditions.

It follows to note, however, that this material (infectious RNA) causes the disease in insects when administered by the intralymph route [7].

The results of our experiments testify that the cell membrane in insect tissue cultures is changed, having become less permeable, as a result of which the large molecules of nucleic acids cannot penetrate into the cell. The possibility is not excluded of the active influence of ribonuclease, which all this time is being liberated by the cells.

Conclusions

1. Preparations of RNA and DNA, isolated by the phenol method from insects infected with the virus of nuclear polyhedrosis, do not cause the development of polyhedra in cells of insect tissue cultures.

2. Pure virus particles, freed of polyhedrosis protein by means of chemical treatment (based on Bergold), being completely infectious for intact insects, when introduced into a culture do not cause the development of the jaundice virus under cultural conditions.

Literature

1. Gershenzon, S. M., Kok, I. P., Vitaye, K. I., et al., Dopovidi AN Ukrainsk RSR, 1960, No 12, p 1638.
2. Gershenzon, S. M., Kok, I. P., Vitaye, K. I., et al., Vopr. Virusol., 1963, No 3, p 334.
3. Miloserdova, V. D., Dopovidi AN Ukrainsk RSR, 1962, No 4, p 540.
4. Bergold, G. H., In the book: Handbuch der Virusforschung, Wien, 1958, Bd 4, S 60.
5. Grace, T. D., Nature, 1962, v 195, p 788.
6. Mirth, L., Lebeurier, G., C. R. Acad. Sci. (Paris), 1962, v 254, p 1495.
7. Puck, T. T., Cieciura, S. J., Fishor, H. W., J. exp. Med., 1957, v 106, p 145.
8. Vaughn, J. L., Faulkner, P., Virology, 1963, v 20, p 484.